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Research Article

A COMPARATIVE STUDY INDICATES METHIMAZOLE INDUCED CHEMICAL HYPOTHYROIDISM CAUSES INHIBITION OF PINEAL GLAND KARYOMORPHOLOGY IN THREE DIFFERENT SPECIES OF ANIMALS

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ABSTRACT

In the present investigation, influence of experimentally induced hypothyroidism on pineal karyomorphology was studied in three different species of animals – post pubertal male mice (*Mus musculus*), adult male rats (*Rattus rattus*) and neonatal male chicks (*Gallus domesticus*). Twenty-two animals of each species group were used, divided into two sets of experimental groups, Group A the control group and the other Group B the treated group. In Group A, eleven control animals of each species were given normal pelleted feed and normal drinking water ad libitum. In GroupB, the adult male mice (N=11), rats (N=11) and chicks (N=11) were supplied with normal pelleted feed and methimazole dissolved in drinking water (at a dose of 1 gm/l). After expiry of the experimental period of fifteen consecutive days each set of control and treated animals were divided into two groups and killed by etherisation. In the first set of control (N=6) and treated (N=6) experiments, animals were considered for histological studies. The second set of control (N=5) and treated (N=5) experiments, animals were considered for T_4 assay. Present data reveal that methimazole caused a significant reduction reaching about detection value of serum T_4 (µg/dl) levels in all the three species of animals. Such hypothyroid animals showed inhibition of the pineal gland activity as seen from significantly decreased pinealocyte nuclear diameter (µm) in all three species of animals, along with an increased pinealocyte nuclear density per microscopic field in mice and rats. Our study indicates that methimazole induced chemical hypothyroidism inhibits pineal gland activity in both mammals and birds.

Keywords: Methimazole, T_4, hypothyroidism, pinealocyte nuclear diameter, mice, rat, chick.

INTRODUCTION

The pineal gland surrounded by its mystic nature has often gathered speculation regarding its relationship with other peripheral endocrine organs and enormously attracted the interest of several group of pinealogists (Mess and Peter, 1975). Amongst all its relationship with thyroid has been a subject of controversy and no general agreement exists regarding the relationship between pineal and thyroid gland function (Sinha and Chakraborty, 2010).

Interestingly the pineal gland responsiveness towards chemical hypothyroidic status yet remains to be elucidated from a comparative basis. However, a few investigations have indicated that pineal gland metabolism could be altered through thyroid manipulations in mammals but as yet none has been reported from birds.

Evidences have suggested that surgical thyroidectomy decreased pineal melatonin content in hamster and rat during peak pineal melatonin synthesis (Johnson, 1982; Vriend, 1983). Additionally, Reiter and his colleagues from an *in vivo* study concluded that surgical thyroidectomy depressed night time rise of melatonin content in rat (Reiter *et al*., 1982).

A recent study on the karyomorphological status of the pinealocytes have shown that thyroidectomy depressed pineal gland activity which was clearly seen by a significant decrease of pinealocyte nuclear diameter followed by an increased numerical density of pinealocytes in a microscopic field in rat (Sinha and Chakraborty,

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2010). Further supports to these results are that antithyroid agents like thiouracil decreased pineal melatonin in rat (Nir and Hirschmann, 1978).

However, contradictory reports are available which reported that thyroidectomy failed to show an effect on the number of dense core vesicles in pineal cells (Karasek, 1981; Karasek and Stephens, 1981).

To summarize many of the previous results from both *in vitro* and *in vivo* studies it may be emphasized that information on effect of chemical hypothyroidism awaits in depth study at pineal cytophysiological level. Interestingly, the influence of methimazole a potent antithyroidic agent has never been studied to assess its effect of chemical hypothyroidism on pinealocyte karyomorphology in mammals and birds.

Methimazole (1-methyl-2-mercaptoimidazole) has shown to induce chemical hypothyroidism and inhibit production of thyroid hormones. Thus, methimazole, a thioureylene agent inhibits the formation of thyroid hormones by preventing incorporation of iodine into tyrosine residues of thyroglobulin. This is done by interfering with the oxidation of iodide ion and iodotyrosyl groups through inhibition of thyroperoxidase (TPO) enzyme (King *et al*., 1974; Chiasson *et al*., 1979; Chopra *et al*., 1982; Cooper, 1984 and Leung *et al*., 1985).

It has also been proposed that the therapeutic effects of methimazole have been described to its ability to decrease thyroid hormone production. Results have shown that as reported previously chemical hypothyroidism induced with methimazole occurs irrespective of species nature (Kim *et al*., 2001; Rosebrough and McMurtry, 2003; Rosebrough *et al*., 2009).

In view of such it is felt that a comprehensive comparative study of pinealocyte karyomorphological activity under methimazole induced chemical hypothyroidism condition appears to be convincingly rewarding in view of pineal status in relation to hypothyroidic condition *in vivo*.

In the current endeavour, influence of methimazole induced chemical hypothyroidism associated with non-detectable T_4 level studied on pinealocyte karyomorphological activity from a comparative view using three different species namely mice (*Mus musculus*), rat (*Rattus rattus*) and chick (*Gallus domesticus*).

MATERIALS AND METHODS

Postpubertal 45 day old male mice (Charles Foster strain) of body weight 20-22 g, 60 day old male albino rat of body weight 55-65 g, and neonatal 15 day old male chicks (body weight 70-80 g) were used for the experiments. The animals were housed in different photoperiodic chambers and acclimatized to laboratory conditions for three days prior to experimental use. During this period and during the entire experimental schedule, they were exposed to a daily photoperiod of 12 L : 12 D. The lights controlled by timer switches (Superswitch, Surrey, U.K.) were on at 06.00 Hrs. and switched off at 18.00 Hrs. The animals were supplied with standard pelleted feed and water *ad libitum*. A total of sixty-six animals, twenty-two of each group were used. Each group of twenty-two animals was again divided into two sets of experiments – one control group A and the other treated group B. All the experimental animals were maintained and used as per guidelines of Institutional Ethics Committee, University of Calcutta, accredited by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India.

Control: Adult mice (N = 11), male rats (N = 11) and chicks (N = 11) constituting the control group were supplied with standard pelleted feed and normal tap water for drinking daily for 15 consecutive days i.e. till the last day of the experiment. They were not subjected to any other treatment.

Methimazole treatment: Comparable to the control group, the adult male mice (N = 11), rats (N = 11) and neonatal chicks (N = 11) were supplied with normal pelleted feed and tap water in which methimazole powder was dissolved instead of normal tap water. For every 1 litre of tap water 1 gm, of methimazole (Sigma, USA) powder was dissolved. This treatment was given for fifteen consecutive days.

After expiry of the experimental period, each set of control and treated animals were divided into two groups and killed by etherisation.

In the first set of control (N = 6) and treated (N = 6) experiments, animals were considered

for histological studies, Pineal gland were excised and fixed in Bouin's fluid and later processed for paraffin embeding and microtomy. Whereas control (N = 5) and treated (N = 5) of each species were used for T_4 assay.

Histology

Nuclear size has long been considered to be a reliable index of cellular activity (Rather, 1958) increase in nuclear size has also been considered to reflect an increase in protein content (Oehlert and Schultze, 1960; Citoler *et al.*, 1965; Edwards and Gray, 1970; Reiter, 1977). The alteration in the nuclear size influences the synthetic and secretory activity of pineal gland in mammals and birds. The nuclear size responds to various physiologically induced changes and reflects glandular activity in several mammalian and avian species. An active phase is characterized by increased pinealocyte nuclear size indicating stimulation of synthesis activity, whereas an inhibitory phase is characterized by decreased nuclear size suggestive of inhibition of gland cells (Quay, 1965, 1976; Chakraborty and Maiti, 1981; Chakraborty *et al*., 1981; Chakraborty and Maitra, 1982; Diehl *et al*., 1984; Sahu and Chakraborty, 1983, 1986; Hira *et al*., 1989; Peschke *et al*., 1989; Martinez Soriano *et al*., 1990; Chakraborty *et al*., 1993; Chakraborty, 1981, 1993, 1994; Chakraborty and Sarkar, 1994; Ganguli *et al.*, 1998; Bandopadhyay and Chakraborty, 2010; Sinha *et al*., 2010; Sinha and Chakraborty, 2010; Bandopadhyay *et al*., 2010a,b,c; Bandopadhyay *et al*., 2011a,b). Also evidences elucidate that thyroid physiology has a regulating effect on the pineal synthetic and secretory activity (Karasek, 1981; Karasek and Stephen, 1981; Johnson, 1982; Reiter *et al*., 1982; Vriend, 1983; Sinha *et al*., 2010; Sinha and Chakraborty, 2010).

Pineal karyomorphology

In view of the foregoing observations, in the present investigation the pineal gland activity had been mainly judged from karyometric values of the pineal parenchymal cells. Investigations were carried out from 5 µm thick sections stained with iron-alum-haemotoxylin and eosin. Thus for morphometric evaluations at least 250 oval to round nuclei were measured from each of the five randomly selected mid sagittal sections per specimen. Unlike oval nuclei, where the mean of the short and long axis were measured, in case of round nuclei only the diameter was measured. In all cases, nuclei were measured under oil immersion using 15 ocular X 100 objective lenses along with ocular micrometer scale. All ocular diameter values were then converted to µm values. Individual values of the specimen were the mean figure of those five sectional measurements. The final mean values of the experimental and control groups were computed form these individual measurements.

Numerical density of pinealocytes per microscopic field

To determine the pinealocytes number (numerical density), per microscopic field, in every specimen five different areas were counted and means were calculated. In this way data was collected from six specimens from each of the control and treated groups of mice and rat (Chakraborty *et al*., 1981).

Hormone Assay

The second set of control (N = 5) and treated (N = 5) experiments animals were considered for T_4 (3,5,3´,5´-L-tetraiodothyronine) assay, using Automated Chemiluminescence System. The ACS 180 ® according to manufactures protocol. In the circulation 99.95% of T_4 is reversibly bound to transport proteins, primarily thyroxin binding globulin (TBG) and to a lesser extent albumin and prealbumin. Unbound or free T_4 is metabolically active and bound T_4 is metabolically inactive, acting as a reserve (Watts and Keffer, 1982; Chattraj and Watts, 1987). The ACS: 180 T_4 assay is a competitive immunoassay using direct chemiluminescent technology. T_4 in the sample competes with T_4, which is covalently coupled to paramagnetic particles in the Solid Phase, for a limited amount of acridinium ester – labeled monoclonal mouse anti – T_4 antibody in the Light Reagent. The system automatically performs the steps and with the input of Master Curve Card values, the system reports the results according to the selected options as described in systems operating instructions. The system reports T_4 results in µg/dL (mass units) or □mol/L (S I Units), depending on the units defined when setting up the assay. The ACS: 180 T_4 assay measures T_4 concentrations up to 30 µg/dL (387nmol/L) with a minimum detectable concentration of 0.5 µg/dL (6.4 nmol/L).

Statistical Analysis

Values were presented as the means of the observations following experimental manipulations. All the karyomorphological and

biochemical values for the control and treated animals were compared and the level of significance was statistically evaluated by Student's "t" test (Winer, 1971) and through ANOVA (using the package Microcal Origin, Version 4.00).

OBSERVATIONS

Histology and Morphometric Study

Control: Light microscopic studies of sections mice (Figure 1) and rat (Figure 2) pineal gland appears almost similar.

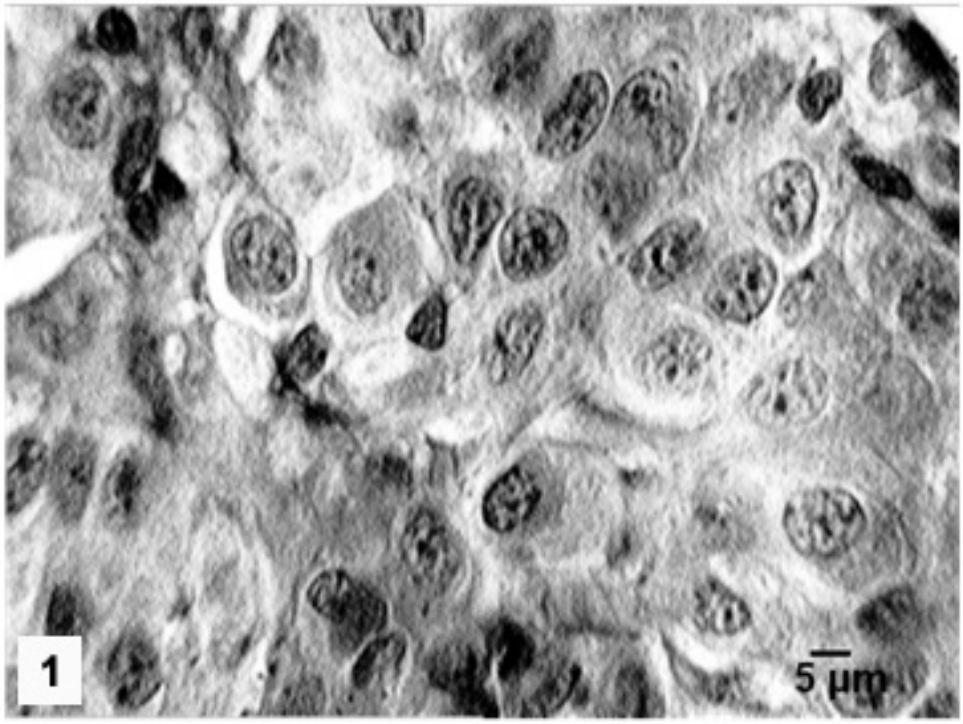

Figure 1. Photomicrograph showing normal pinealocyte morphology in control mice.

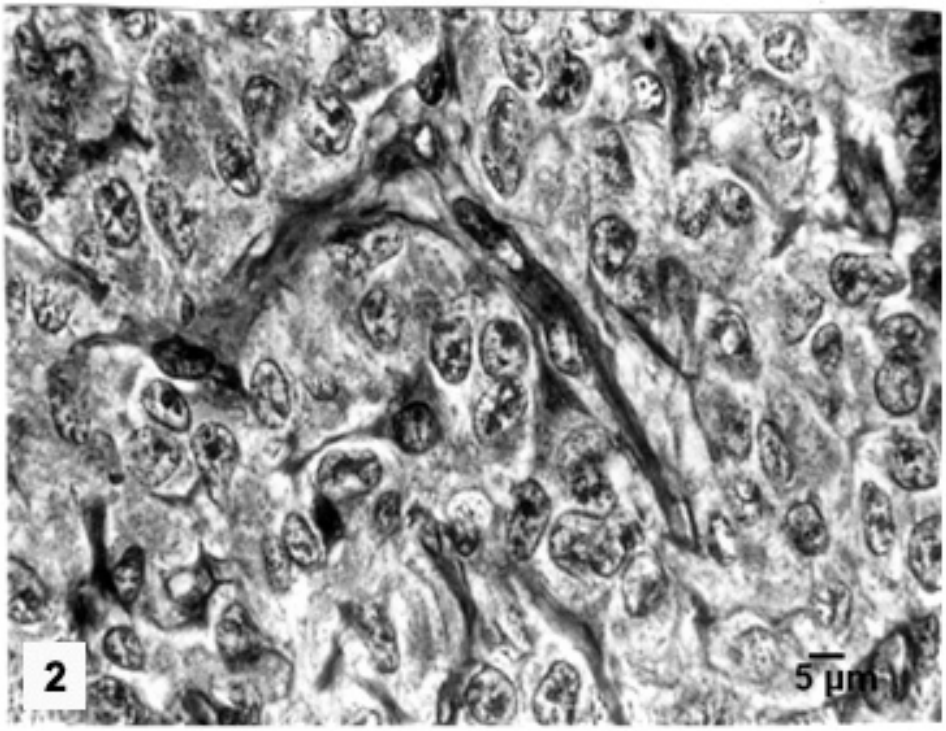

Figure 2. Photomicrograph showing normal pinealocyte morphology in control rat.

The pineal parecnchyma is comprised of pinealocytes of varying size, maximally with round to oval nuclei of different diameters. Most of the nuclei are with single indistinct nucleolus and a few with two to three nucleoli. Generally the parenchyma appears homogenous in nature and the pinealocytes lacked definite orientation. Cell outline of the pinealocyte is not clearly recognizable by conventional histological preparations.

Whereas the pineal gland of chick section (Figure 3) shows three types of pinealocytes. One type is the ependemocytes – the characteristic acidophil pinealocytes with oval nuclei and large columnar cells forming a palisade around the pineal lumen or luminae of follicles. The others are hypendymocytes; peripheral to the former, irregular group of smaller cells and lastly the plasma cells, basophilic cells having large nuclei and resemble neurons in having processes.

Methimazole treatment: In comparison to control group of animals a significant atrophy of the pineal gland was induced by methimazole treatment in mice (Figure 4), rat (Figure 5) as well as in chicks (Figure 6).

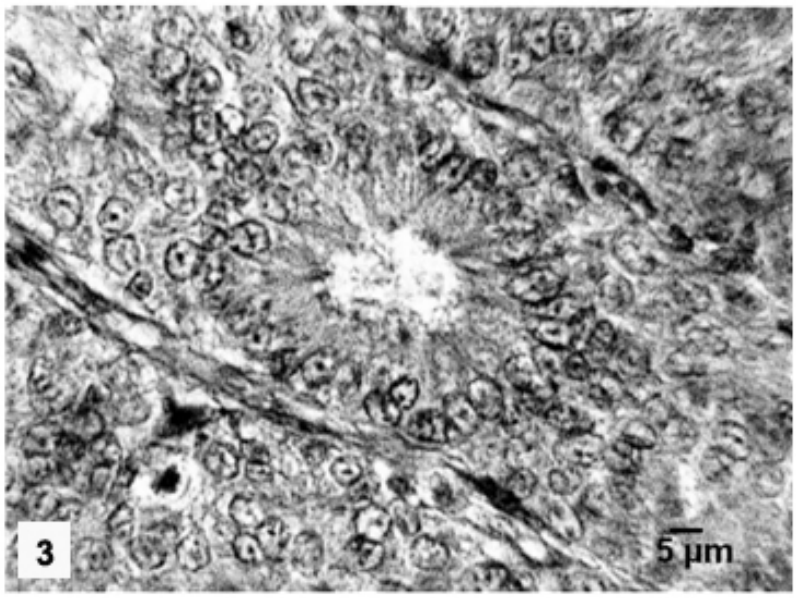

Figure 3. Photomicrograph showing normal pinealocyte morphology in control chick.

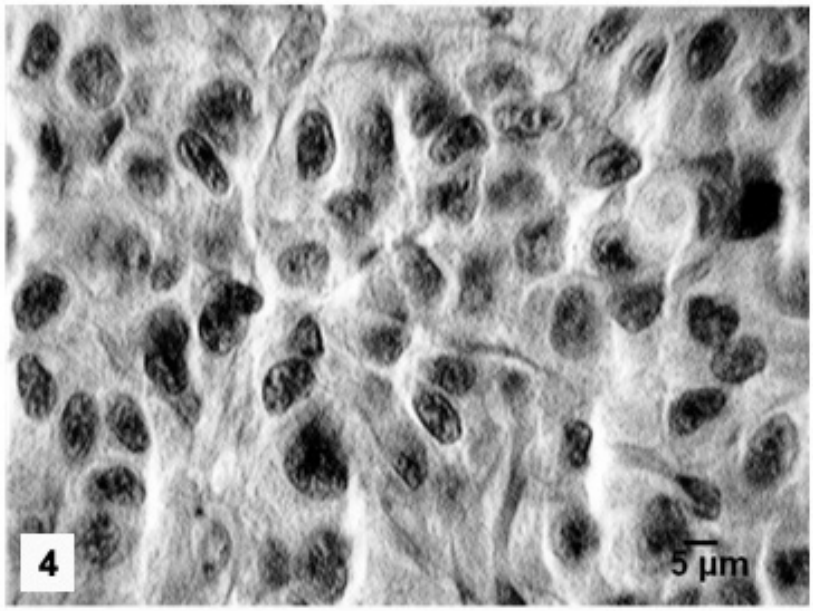

Figure 4. Photomicrograph showing hypotrophied pineal cell nucleus with decreased nuclear diameter in mice following methimazole treatment.

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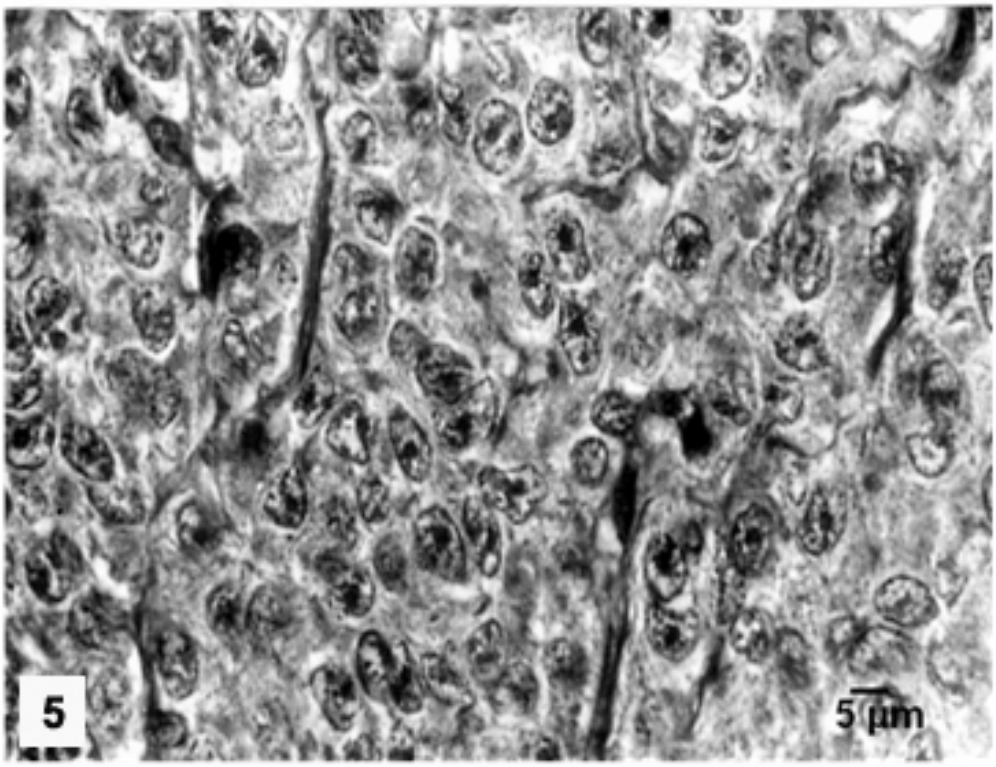

Figure 5. Photomicrograph showing hypotrophied pineal cell nucleus with decreased nuclear diameter in rat following methimazole treatment.

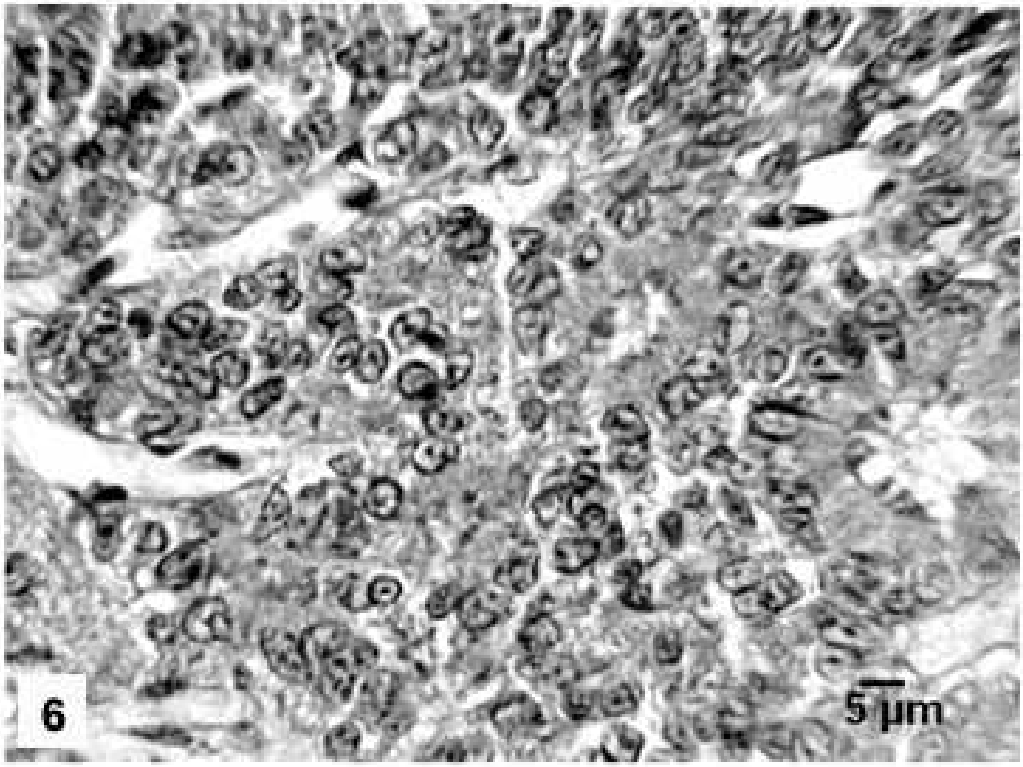

Figure 6. Photomicrograph showing hypotrophied pineal cell nucleus with decreased nuclear diameter in chick following methimazole treatment.

The pineal gland in these animals exhibited smaller pinealocytes when compared to the control. This treatment was found to induce hypoactivity of mice, rat and chick pineal cells. $F(1,10) = 151.041$, $p < 0.001$ in mice, $F(1,10) = 146.66$, $p < 0.001$ in rats and $F(1,10) = 142.15$, $p < 0.001$) in chicks (Figure 7). The nucleus showed indistinct nucleolus and granulated chromatin material. This was supported by the significant increase in numerical density of pinealocytes when compared to the numerical density observed in the pinealocytes of animals belonging to the control group [$F(1,10) = 38.36$, $p < 0.01$ in rats (Figure 8) and $F(1,10) = 46.725$, $p < 0.001$ in mice].

T_4 assay: Methimazole is an antithyroid drug that blocks thyroid hormone synthesis and thus is supposed to cause a concomitant decrease in the circulating plasma T_4. T_4 assay was done to confirm this and results show that in all experimental group of animals serum T_4 has been drastically reduced ($p < 0.001$) in comparison to the control groups (Figure 9).

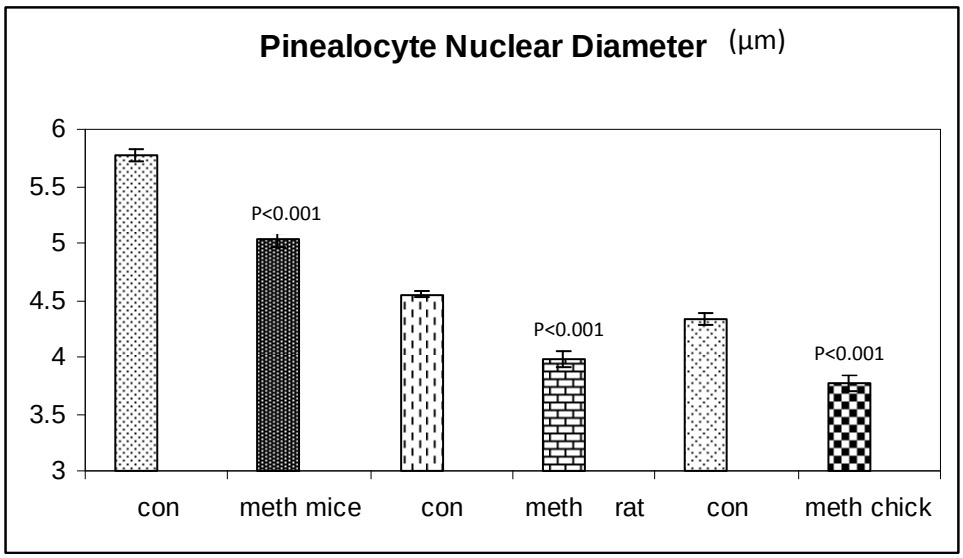

Figure 7. Histogram comparing the effects on karyomorphological values in control and methimazole treated mice, rats and chicks. Note the significantly decreased nuclear diameter values in each species following methimazole treatment.

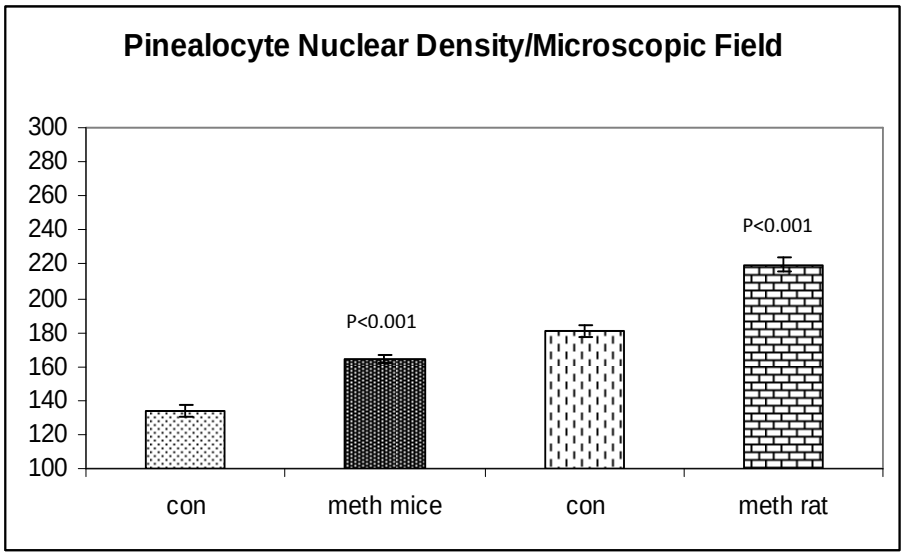

Figure 8. Histogram comparing the pinealocyte nuclear density in control and methimazole treated mice and rats. Note the significantly decreased nuclear density values following methimazole treatment.

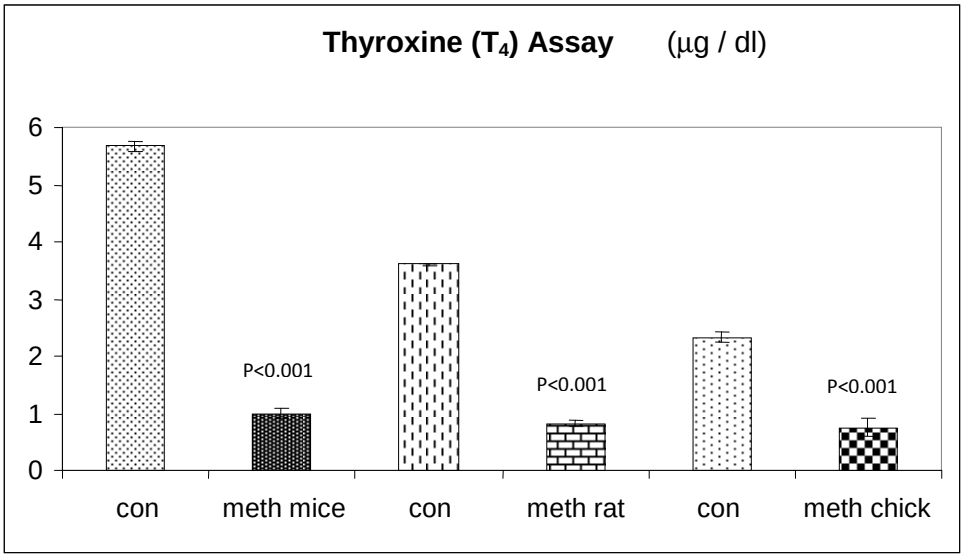

Figure 9. Histogram comparing the T_4 values in control and methimazole treated mice, rat and chick. Note the drastic diminished value of the serum T_4 in methimazole treated groups of each species.

DISCUSSION

The present investigation offers an insight into the morphological and functional organization of the pineal gland in mice, rats and chicks in response to experimental hypothyroidism generated by administration of thyroid-blocker methimazole. Methimazole as we know is a thioureylene agent that inhibits the formation of thyroid hormones by preventing the incorporation of iodine into tyrosin residues of thyroglobulin. This is done by interfering with the oxidation of iodide ion and iodotyrosyl groups through the inhibition of the thyroperoxidase (TPO) enzyme (King *et al*., 1974; Chiasson *et al*., 1979; Chopra *et al*., 1982; Cooper, 1984; Leung *et al*., 1985; Kim *et al*., 2001; Rosebrough and McMurtry, 2003; Rosebrough *et al*., 2009).

Our present study shows that methimazole effectively inhibits the formation of thyroid hormones thereby causing hypothyroidism as is evident from our results of serum T_4 assay. Administration of methimazole caused significant reduction of serum T_4 level in all the three species of animals.

This study further gives emphasis on the cytophysiological behavior of the gland as reflected in concomitant alterations in the karyomorphological values in the glands induced by methimazole. Current study indicates that administration of thyroid blocker methimazole significantly depressed pineal gland activity which was clearly seen from decreased pinealocyte nuclear diameter values with an increased pinealocyte nuclear density per microscopic field in mice and rats. This corroborates well with previous studies where antithyroid agents like thiouracil depressed pineal melatonin in rats (Nir and Hirschman, 1978) and surgical thyroidectormy induced an overall depressant effect on pineal cytomorphology (Sinha and Chakraborty, 2010) as well as melatonin content (Johnson, 1982; Reiter *et al*., 1982; Vriend, 1983).

It may be noted that present studies related to the cytological features included measurement of the nuclear diameter, an indicator of the glandular activity (Oehlert and Schultze, 1960; Citoler *et al*., 1965; Edwards and Gray, 1970; Reiter, 1977; Peschke *et al*., 1989). The alteration in the nuclear size influences the synthetic and secretory activity of pineal gland in mammals and birds. The nuclear size responds to various physiologically induced changes and reflects glandular activity in several mammalian and avian species. An active phase is characterized by increased pinealocyte nuclear size indicating stimulation of synthesis activity, whereas an inhibitory phase is characterized by decreased nuclear size suggestive of inhibition of gland cells (Quay, 1965, 1976; Chakraborty and Maiti, 1981; Chakraborty *et al*., 1981; Chakraborty and Maitra, 1982; Diehl *et al*., 1984; Sahu and Chakraborty, 1983, 1986; Hira *et al*., 1989; Peschke *et al*., 1989; Martinez Soriano

et al., 1990; Chakraborty *et al*., 1993; Chakraborty, 1981, 1993, 1994; Chakraborty and Sarkar, 1994; Ganguli *et al*., 1998, Bandopadhyay and Chakraborty, 2010, Sinha *et al*., 2010, Sinha and Chakraborty, 2010; Bandopadhyay *et al.*, 2010a,b,c; Bandopadhyay *et al*., 2011a,b). Also evidences elucidate that thyroid physiology has a regulating effect on the pineal synthetic and secretory activity (Karasek, 1981; Karasek and Stephen, 1981; Johnson, 1982; Reiter *et al*., 1982; Vriend, 1983; Sinha and Chakraborty, 2010; Sinha *et al*., 2010).

The present study indicates that administration of thyroid blocker – methimazole inducing chemical thyroidectomy with suppressed plasma T_4 level significantly depressed pineal activity, which was clearly seen by decreased pinealocyte nuclear diameter followed by an increase in the numerical density of pinealocytes in a microscopic field in rats and mice as compatible with our observation that hypothyroidism induced an overall depressant effect on pineal cytomorphology.

However, some earlier contradictory results have shown that thyroidectomy had no effect on pineal ultrastructure (Karasek, 1981; Karasek and Stephen, 1981). In essence, the present study suggests that methimazole induced thyroidectomy showed similar effects as regards suppression of pineal gland activity in mice (*Mus musculus*), rat (*Rattus rattus*) and chick (*Gallus domesticus*) in line with that observed earlier after surgical thyroidectomy in rat (Sinha and Chakraborty, 2010).

CONCLUSION

In conclusion, our comparative study of pineal gland karyomorphology indicates that irrespective of the species, methimazole causes chemical hypothyroidism and equally causes inhibition of pineal gland karyomorphology as assessed from morphometric and hormonal studies.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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